Bol. Inst. Esp. Oceanogr. 15 (1-4). 1999: 485-488 **BOLETÍN** INSTITUTO ESPAÑOL DE OCEANOGRAFÍA
ISSN: 0074-0195

Levels of copper, zinc, manganese and iron in two fish species from salt marshes of Cadiz Bay (southwest Iberian Peninsula)

J. M. Arellano[1], *J. B. Ortiz*[2], *D. Capeta Da Silva*[1], *M. L. González de Canales*[2], *C. Sarasquete*[1] *and J. Blasco*[1]

[1] Instituto de Ciencias Marinas de Andalucía (CSIC). Polígono del Río San Pedro, s/n. 11510 Puerto Real (Cádiz), Spain
[2] Departamento de Biología Animal, Vegetal y Ecología. Facultad de Ciencias del Mar. Universidad de Cádiz. Polígono del Río San Pedro, s/n. 11510 Puerto Real (Cádiz), Spain

Received October 1997. Accepted April 1998.

ABSTRACT

The authors analysed copper, iron, manganese and zinc concentrations in liver, gills and muscle in two species of fish from salt marshes on Cadiz Bay: the sole *Solea senegalensis* (Kaup, 1858) and the killifish *Fundulus heteroclitus* (Linnaeus, 1758). The highest concentrations of copper and iron, in both species, were found in the liver. The mean values reported were: 977.71 ± 42.92 and 354.42 ± 22.80 μg g^{-1} for iron and 124.16 ± 15.97 and 60.17 ± 1.95 μg g^{-1} dry weight for Cu in *S. senegalensis* and *F. heteroclitus*, respectively. The distribution pattern of zinc in organs was different in both fish, so *S. senegalensis* showed the highest values in liver and *F. heteroclitus* in gills. The results of the present study enabled us to determine the background concentrations of these metals in both species and their distribution in the different organs. These data will constitute a reference for future studies on the evolution of contamination in this area.

Key words: Copper, iron, manganese, zinc, fish, liver, gills, muscle, Cadiz Bay.

RESUMEN

Concentraciones de los metales cobre, hierro, manganeso y zinc en peces de las marismas de la bahía de Cádiz (suroeste de la península Ibérica)

Se han analizado las concentraciones de los metales cobre, hierro, manganeso y zinc en branquias, músculos e hígado en ejemplares de dos especies de peces: Solea senegalensis *(Kaup, 1858) y* Fundulus heteroclitus *(Linnaeus, 1758) recolectados en las marismas de la bahía de Cádiz (suroeste de la península Ibérica). Las concentraciones más elevadas para hierro y cobre, en ambas especies, se encontraron en el hígado. Los valores medios registrados fueron: 977,71 ± 42,92 y 354,42 ± 22,80 μg* g^{-1} *en el caso del Fe, y 124,16 ± 15,97 y 60,17 ± 1,95 μg* g^{-1} *peso seco para el Cu en* S. senegalensis *y* F. heteroclitus, *respectivamente. El comportamiento del zinc fue diferente en ambas especies, ya que mientras en* S. senegalensis *el hígado registró los valores más elevados, en* F. heteroclitus *éstos se hallaron en branquias. Los resultados obtenidos permiten determinar la concentración basal de estos metales en estas especies y su distribución en los órganos analizados, y constituyen una referencia en estudios posteriores de la evolución de la contaminación en esta zona.*

Palabras clave: *Cobre, hierro, manganeso, zinc, pez, hígado, branquias, músculos, bahía de Cádiz.*

INTRODUCTION

Contamination of the marine environment by metals has risen in recent years due to the global population increase and industrial development. The littoral and estuarine zones are more exposed to this problem than the oceans because of their proximity to the sources of pollution.

The distribution of heavy metals in the different components of the marine ecosystem (water, sediment, flora, fauna) is regulated by physico-chemical processes, e.g. dilution, diffusion, precipitation and sorption, as well as other processes involving marine organisms, such as uptake and elimination.

Biota is recognised as an important indicator of heavy-metal contamination of the marine environment. Fish are used as indicators of marine pollution because they are relatively large and easily identified. Baseline surveys of the concentration of heavy metals in selected fish species have been conducted on several coasts around the world (Essink, 1989; Hornung and Kress, 1991; Pocklington and Wells, 1992; Otway, 1992; Rainbow and Phillips, 1993; Sharif *et al.,* 1991; Turgeon and O'Connor, 1991; Vukadin, Stegnar and Smodis, 1982).

The salt marshes around Cadiz Bay constitute an area supporting a high level of aquaculture production, mostly fish, molluscs and crustaceans. However, urban and industrial sewage are discharged in this zone, threatening the water quality and the viability of aquaculture.

The objective of the present paper was to evaluate the utility of different organs and tissues (i.e. liver, gills and muscle) in two fish species, the sole *Solea senegalensis* (Kaup, 1858) and the killifish *Fundulus heteroclitus* (Linnaeus 1758), as indicators of heavy-metal contamination. The authors also created a database for further studies on the evolution of contamination. These species were selected because they have different habitats and feeding habits, and could be representative of different sources of heavy metals.

MATERIALS AND METHODS

The species were collected between October and February in the salt ponds of Cadiz Bay (southwest Iberian Peninsula). Afterwards, they were transported in aerated tanks to the laboratory and kept in running seawater (3 or 4 days) until they were anaesthetised with MS-222 and dissected out. Pools five (liver, gills and dorsal muscle) from four to five specimens were prepared. The size of *F. heteroclitus* ranged between 6.3-11.7 cm and the weight between 3.5 and 12.4 g; *S. senegalensis* had a mean size and weight of 36.5 ± 3.2 cm and 435 ± 62.1 g. Samples were freeze-dried and digested according to the method of Amiard *et al.* (1987). Heavy metals were analysed by atomic flame absorption spectroscopy (Perkin-Elmer, 3110) and the analytical procedure was checked using reference material (DORM1, Institute of Environmental Chemistry, NRC Canada). Heavy-metal concentrations are expressed as $\mu g\ g^{-1}$ dry weight. Statistical analysis of results was carried out by a three-way analysis of variance.

RESULTS AND DISCUSSION

Figures 1 and 2 show mean values and standard deviations for Cu, Fe, Mn and Zn concentrations in the liver, gills and muscle of *S. senegalensis* and *F. heteroclitus.*

The variance analysis showed differences in relation to the studied organ ($P < 0.05$). A range multiple (Tukey-Kramer) was carried out, and differences among liver and muscle and gills were observed for the four metals. However, the differences were not significant between gills and muscle ($P > 0.05$).

In both species, the highest Cu and Fe values were found in the liver. The liver plays an important role in metal sequestration, because heavy metals may be bound to metallothioneins (Cu, Zn, Cd) or other ligands (Dallinger, 1995). Gills showed the highest values for Mn. A different pattern was observed in both species for Zn levels. In *Solea senegalensis,* the sequence was liver > gills > muscle, whereas in *F. heteroclitus* it was gills > muscle > liver. The liver of *S. senegalensis* showed the highest values, with a ratio $[M]_{Solea}/[M]_{Fundulus}$ of 2.75 for Fe and 1.30 for the other metals. The accumulation of heavy metals in the liver is associated with low levels of contaminants. When organisms are exposed to high metal concentrations, the liver cannot regulate the hepatic levels, and an increase is observed in other tissues (Benedetti, Albano and Mola, 1988). Copper and Fe concentrations in gills and muscle of *S. senegalensis* were lower than in *F. heteroclitus.* For muscle, all of the heavy metals analysed

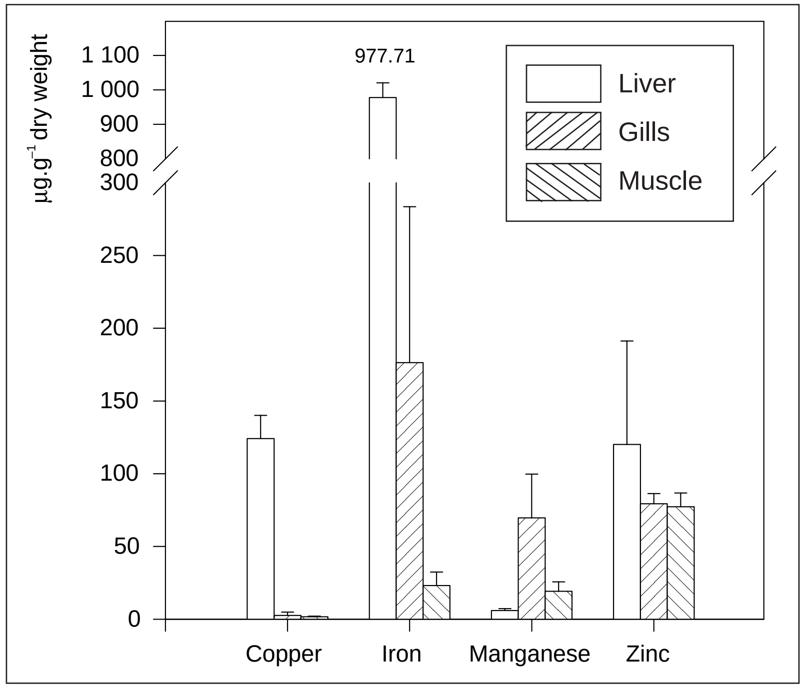

Figure 1. Concentrations of Cu, Fe, Mn and Zn (mean ± standard deviation) in liver, gills and muscle of *S. senegalensis*

showed higher concentrations in *F. heteroclitus* than *S. senegalensis* This pattern could be related to these species' different habits, their biology, physiology or feeding; *F. heteroclitus* is an active organism with a higher metabolism rate than *S. senegalensis,* which is a benthic species with sedentary habits. Therefore, the need for Cu and Zn in the muscle of *F. heteroclitus* could be higher than for *S. senegalensis.*

Heavy metals showed significant differences among their concentrations ($P < 0.05$). Thus, Fe background levels were different from those of the other metals. Levels of copper and zinc were also different; while in the liver these values were similar. With regard to the species factor, the differences were significant ($P < 0.05$).

The Cu concentration found in the muscle of *S. senegalensis* (an edible fish) did not exceed the max-

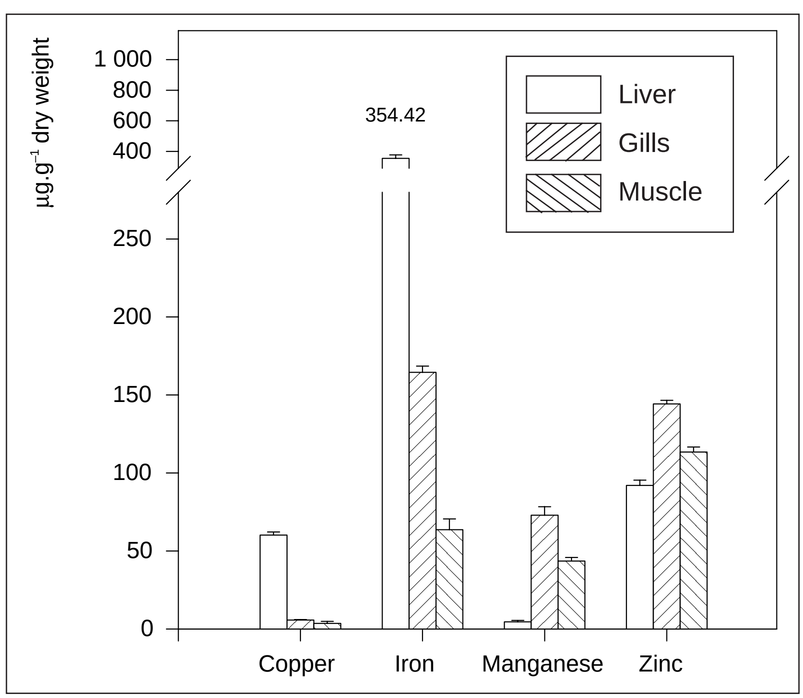

Figure 2. Concentrations of Cu, Fe, Mn and Zn (mean ± standard deviation) in liver, gills and muscle of *F. heteroclitus*

imum legal limit in Spain (Cu: 20 µg g^{-1} wet weight). Copper levels in this tissue were similar to those reported by Cossa *et al.* (1992) in flounder, *Platichthys flesus* (Linnaeus, 1758), and Stenner and Nickles (1975) in *Solea solea* from uncontaminated sites. The use of copper sulphate in aquaculture as an alternative to antibiotic treatment (Sarasquete and Gutiérrez, 1987) could increase the levels of this metal and reduce water quality.

The selected species present different feeding habits and habitats. Therefore, the study of their metal levels is very interesting, because these concentrations provide information about the main routes of heavy-metal uptake. *S. senegalensis* is a benthic species which lives on the bottom, and these concentration can indicate the bioavailability of heavy metals from the sediments (Luoma, 1983). On the other hand, *F. heteroclitus* is a non-migratory species which lives in estuary zones and is prey to *Dicentrachus puntactus* (Bloch, 1792), *D. labrax* (Linnaeus, 1758), *Sparus aurata* (Linnaeus, 1758) and *Anguilla anguilla* (Linnaeus, 1758). Consequently, the metal levels in this species could contribute to an enhancement of metal concentrations in commercial species. However, none of the metals studied in the present research project are non-biomagnifiable.

This paper is a first step in the study of the heavy-metal levels in fishes, and will be completed by the analysis of metals in different compartments of the ecosystem (water, sediment and other organisms which live on the bottom and in the water column), and the effect of gender, size and age on heavy-metal concentrations.

REFERENCES

Amiard, J. C., A. Pineau, H. L. Boiteau, C. Metayer and C. Amiard-Triquet. 1987. Application de la spectrometrie d'absorption atomique Zeeman aux dosages de huit elements traces (Ag, Cd, Cr, Cu, Mn, Ni, Pt et Se) dans des matrices biologiques solides. *Water Res.* 21 (6): 693-697.

Benedetti, I., A. G. Albano and L. Mola. 1988. Histomorphological changes in some organs of the brown bullhead, *Ictalurus nebulosus* Lesueur, following short-and long-term exposure to copper. *J. Fish Biol.* 34: 273-280.

Cossa, D., D. Auger, B. Averty, M. Lucon, P. Masselin and L. Nöel. 1992. Flounder (*Platichtys flexus)* muscle as indicator of metal organochlorine contamination of French Atlantic coastal waters. *Ambio* 21: 176-182.

Dallinger, R. 1995. Metabolism and toxicity of metals: metallothioneins and metal elimination. In: *Cell Biology in Environmental Toxicology*. M. P. Cajaraville (ed.): 171-190. Univ. del País Vasco. Bilbao.

Essink, K. 1989. Chemical monitoring in Dutch Wadden Sea by means of benthic invertebrates and fish. *Helgoländer Meerseumters* 43: 435-446.

Hornung, H. and N. Kress. 1991. Trace elements in offshore and inshore fish from the Mediterranean coast of Israel. *Toxicol. Environ. Chem.* 31-32: 135-145.

Luoma, S. N. 1983. Bioavailability of trace metals to aquatic organisms a review. *Sci. Total Environ.* 28: 1-22.

Otway, N. M. 1992. Bioaccumulation studies on fish: choice of species, sampling designs, problems and implications for environmental management. In: *Proceedings of Bioaccumulation Workshop: Assessment of distribution, impacts and bioaccumulation of contaminants in aquatic environments.* Water Board Australian Science Association Incorparation: 103-113. Sydney.

Pocklington, P. and P. G. Wells. 1992. Polychaetes. Key taxa for marine environmental quality monitoring. *Mar. Pollut. Bull.* 24: 593-598.

Rainbow, P. S. and D. J. H. Phillips. 1993. Cosmopolitan biomonitors of trace metals. *Mar. Pollut. Bull.* 26: 593-601.

Sarasquete, C. and M. Gutiérrez. 1987. Protozoosis en piel de anguilas, *Anguilla anguilla* L. 1758 *(Osteichthyes, Anguillidae)* en un cultivo intenso. *Cuadernos Marisqueros Publicación Técnica* 12: 655-658.

Sharif, A. K. M., A. I. Mustafa, A. H. Mirza and S. Safiullah. 1991. Trace metals in tropical marine fish from the Bay of Bengal. *Sci. Total Environ.* 107: 135-142.

Stenner, R. D. and G. Nickless. 1975. Heavy metals in organisms of the Atlantic coast of S.W. Spain and Portugal. *Mar. Pollut. Bull.* 6 (6): 89-92.

Turgeon, D. D. and T. P. O'Connor. 1991. Long Island Sound: Distributions, trends and effects of chemical contamination. *Estuaries* 14: 279-289.

Vukadin, I., P. Stegnar and B. Smodis. 1982. Fate and distribution of toxic heavy metals in sediments and organisms of the Kastela Bay. *Acta Adriat.* 23: 307-312.